REMARKS

Claims 12-22 are now pending. A "clean" version of the amendment to the specification and the amended claim set are provided above. Amendments made to the specification and the claims are indicated in the section entitled "Version Showing Changes Made", which follows these remarks.

The Invention

The present invention relates to methods for analyzing nucleic acid samples for sequence variation at multiple loci. In a preferred embodiment, the invention provides a method for analyzing a nucleic acid sample comprising three or more loci, each locus having at least two different allelic sequences. In this embodiment, at least three pairs of oligonucleotide probes are hybridized to the target nucleic acid. One member of each oligonucleotide pair is coupled to a FRET donor, while the other is coupled to a FRET acceptor, wherein each of the FRET acceptors have a different emission spectrum. Each pair is hybridized to the target in such a manner that the donor and acceptor are brought near enough to generate an emission signal from said FRET acceptor. Furthermore, at least one member of each oligonucleotide pair has a sequence that will allow for differential hybridization depending on the sequence present at the locus of hybridization. Differential hybridization is accomplished by repeating the hybridization at a second temperatures such that the pair that most strongly hybridizes to the target is indicated.

Restriction Requirement

Original Claims 1-45 are subject to a restriction requirement, wherein the Examiner has identified the following three inventions:

I. Claims 1-22, directed to a method of analyzing a nucleic acid;

II. Claims 23-40, directed to a method of analyzing a nucleic acid; and

III. Claims 41-45, directed to a device for mutlichannel color analysis.

Applicants hereby confirm election of Group I, namely Claims 1-22. Applicants reserve the right to pursue patent protection for all non-elected claims in future applications.

Rejections to Canceled Claims

As Claims 1-11 and 23-40 have been canceled without prejudice to Applicants' rights to pursue the subject matter of these claims in one or more continuation, continuation-in-part or divisional applications, withdrawal of the various rejections directed to those Claims is kindly requested.

Rejections Under 35 U.S.C. § 112

Claims 12-22 stand rejected under 35 U.S.C. § 112 as vague and indefinite. The Examiner asserts that "it is unclear how the differential hybridization is corresponding to the emission of said FRET acceptors at different temperatures which provide an indication of the alleles present at different loci (or multiple loci)." It appears that this statement includes a typographical error rendering it unclear, applicant therefore kindly requests clarification.

If the Examiner's statement relates to how emission of FRET acceptors attached to probes can be used to determine the allele present at specific loci, the examiner is directed to pages 18 to 21 of the specification for a detailed discussion. Summarized briefly, one embodiment of the invention includes pairs of probes, one member of each pair having a FRET donor and one having a FRET acceptor, which are hybridized to a specific locus on a target nucleic acid. Each of the FRET acceptors has a different emission spectrum. The hybridization of the probes is designed to bring the donor and acceptor sufficiently near to generate an emission signal. Generation of the emission signal may not require full complementarity between the probes and the target nucleic acid, depending on the hybridization conditions, but with higher levels of complementarity comes stronger hybridization. By performing this hybridization over a range of temperatures and comparing such data with that gathered for other pairs of probes, the pair corresponding to the sequence of a particular allele at a specific locus can be determined.

Examples of this type of analysis can be found in Figures 1 and 9. Figure 1 shows melting curves for genotyping the homozygous wild type, heterozygote, and homozygous mutant for a common mutation in the methylenetetrahydrifolate gene. In this example, a difference in melting temperature of 3°C is observed between the oligonucleotide pair that

corresponds to the target sequence and the oligonucleotide pair that does not.

Figure 9 shows a schematic representation of multicolor hybridization probe genotyping at the apolipoprotein E locus. In this example two pairs of oligonucleotide probes are used to differentiate between the $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ alleles. The first pair contains an oligonucleotide probe complementary to the T at codon 112 and is labeled with fluorscein. The other member of that pair is labeled with FRET acceptor Red 705. The second pair has one oligonucleotide probe complementary to the T at codon 158 and is also labeled with fluroscein. The other member of that pair, however, is labeled with FRET acceptor Red 640, which has a different emission spectrum as compared to Red 704. The fluorscein labeled probes will form destabilizing A:C mismatches when hybridized to the $\epsilon 4$ allele at codon 112, or the $\epsilon 3$ allele at codon 158, thus allowing discrimination between the various alleles.

Claim 15 stands rejected under 35 U.S.C. § 112 as vague and indefinite. The Examiner maintains that it is unclear what is a member of two different probe pairs. Claim 15 has been amended to clearly state what is a member of two different probe pairs, therefore withdrawal of this rejection is kindly requested.

Claims 12-22 stand rejected under 35 U.S.C. § 112 as vague and indefinite. The Examiner asserts that the language "each of the members of said pairs being capable of hybridization in proximity to each other within a segment of said nucleic acid comprising at least one of said multiple loci" makes the Claims unclear whether or not each of the members of said pairs hybridizes to each other. Claim 12 has been amended to clarify that said pairs hybridize to a segment of the nucleic acid to be analyzed, therefore withdrawal of this rejection is kindly requested.

Applicants submit that the application is now in form for allowance and respectfully request early notification of such a finding. If the Examiner believes that there are remaining issues that may be resolved by telephone, she is invited to call the undersigned attorney at (415) 781-1989.

Respectfully submitted,

FLEHR, HOHBACH, TEST,
ALBRITTON & HERBERT

Dated: 1/16/02

Richard F. Trecartin
Reg. No. 31,801

Four Embarcadero Center
Suite 3400
San Francisco, CA 94111-4187
Telephone: (415) 781-1989
1071197

a